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FURTHER STUDY ON EXPERIMENTAL CHOLERA-CARRIERS *

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The early investigations concerning cholera infection in animals were undertaken with a view to establishing the etiologic relation between Koch's comma bacillus and the disease known as Asiatic cholera.

The feeding experiments of Thiersch[1] on mice, Richards on hogs,[1] Wiener[2] on cats, and Karlinski[3] on dogs have not been successfully repeated. Nikati and Rietsch[4] asserted that by injection of cholera vibrios into the intestine of guinea-pigs they had produced a disease resembling Asiatic cholera in man. R. Koch[1] fed cholera cultures to guinea-pigs after having introduced a considerable amount of sodium bicarbonate into the stomach to eliminate the action of its acid. The animals also received opium intraperitoneally. According to Koch, they died of cholera infection. Thomas[1] found that intravenous injection of small amounts of cholera culture killed rabbits; cholera vibrios were found in the intestine, and the animals exhibited pathologic lesions in the intestine which resembled those in fatal human cases. Issaeff and Kolle[5] confirmed these findings, being particularly successful when using young rabbits. Metschnikoff,[1] who infected suckling rabbits by feeding cholera cultures, stated that healthy rabbits brought into close contact with the infected animals died of cholera infection.

The experiments of Violle[6] have a decided bearing on the present question. Violle studied the immunity which results from inoculation of cholera vibrios and other bacteria into the gall-bladder with ligated cystic duct. Vibrios were found in the ligated gall-bladder up to the 15th day after inoculation. The resulting immunity was considerable.

In a previous communication the results of experiments were published which showed the relative values of various methods tried in our endeavor to make cholera-carriers of guinea-pigs. The duration of the state of parasitism following intravesicular inoculation, the distribution of cholera vibrios throughout the alimentary canal, and the elimination of cholera vibrios in the feces of the experimental cholera-

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[1] Cited from Kolle-Schürmann, Kolle and Wassermann's Handb. d. pathogen. Mikroorganismen, 1912, 4, p. 1.

[2] Centralbl. f. Bakteriol., 1896, 19, pp. 205, 595.

[3] Ibid., 1896, 20, p. 150.

[4] Deutsch. med. Wchnschr., 1884, 10, p. 634. Semaine méd., 1884, 4, p. 393.

[5] Ztschr. f. Hyg. u. Infektionskrankh., 1894, 18, p. 17.

[6] Ann. de l'Inst. Pasteur, 1912, 26, pp. 381, 467.

carriers were studied. Further investigations bearing on the nature of this condition are recorded here. In the course of our experiments it became desirable to perform repeated intravenous injections, a procedure hardly practicable with guinea-pigs. Rabbits were substituted, with a consequent necessity of having to repeat on rabbits some of the experiments already published, in order to compare the findings with those obtained in the case of guinea-pigs.

TECHNIC

The technic used here was essentially the same as that used previously. The intravesicular inoculation alone was employed. Blood was obtained by bleeding the guinea-pigs from the carotid artery. The peritoneal cavity was examined for presence or absence of cholera vibrios in the following way: The cavity was opened with strictly aseptic precautions and washed out with 1 c.c. of peptone water, which was then planted into a tube containing peptone water. About half of the spleen was crushed by means of a sterile forceps, and a piece of the left lobe of the liver was cut into small pieces; both were then planted in peptone water. The pleural cavity was opened separately, and a part of the left lower lobe of the lung was planted in the same way as the spleen and the liver. Thus the cultures from peritoneum, liver, lung, and spleen, and the blood cultures, were made before the alimentary canal proper was opened.

EXPERIMENTS ON GUINEA-PIGS

In our first experiments the animals remained apparently in good health after the intravesicular inoculation. At autopsy the guinea-pigs showed no particular changes of the internal organs which could not be explained as due to the surgical encheiresis. The gall-bladder, which was of normal size, contained bile of normal appearance, except in a few instances in which the bile was flaked with white.

In the course of further experiments a culture freshly isolated from an animal's gall-bladder was used for the inoculation of the next series of animals. Thus an intermittent passage through guinea-pigs' gall-bladders and ox-bile culture medium was carried on. The results of inoculation into the gall-bladder of the same strain of cholera vibrio after several passages through animals' gall-bladders differed from those in the earlier experiments. Most of the guinea-pigs which were inoculated with a culture previously passed through several animals were decidedly ill after the inoculation, some dying in from 3 to 4 days after inoculation.

There was a more or less extensive swelling over the abdomen, due to hemorrhagic edema. Numerous cholera vibrios were found in smears and cultures from the edema. The peritoneum was congested and fibrinous exudate covered the surface of the liver and the omentum. The internal organs were congested, the large soft spleen dark-red in color. Microscopic examination of the exudate revealed comparatively few, mostly phagocytosed, vibrios. Cholera vibrios were recovered by culture from the internal organs, peritoneum, blood, and subcutaneous edema. In these cases the infection of the peritoneum and the subcutaneous tissue, injured by operation, was due no doubt to leakage of a small quantity of the cholera culture during the inoculation. Most of the animals survived, and autopsy performed on them later revealed far more extensive changes of the gall-bladder than those noticed in our earlier experiments. The gall-bladder was rather small, and its peritoneal cover showed considerable congestion. It contained a small amount of thick bile of light-yellow color, which under the microscope was found to consist of amorphous debris, desquamated epithelial cells, and leukocytes in various stages of degeneration. As a rule, typical vibrios, isolated or in clusters, were seen in the smears.

The liver in some cases exhibited pronounced gross pathologic lesions. Small yellowish foci resembling miliary abscesses were scattered throughout the liver tissue, forming confluent areas in some instances. The spleen was slightly enlarged, but firm. Smears made from the diseased parts of the liver revealed the presence of typical vibrios.

Examination of Microscopic Sections.—The gall-bladder exhibited various degrees of inflammation. In some cases the epithelium was well preserved, the mucous membrane showing only a slight degree of cellular infiltration. In other cases the epithelium was fairly preserved, but the surface of the mucous membrane was covered with numerous pus cells. The tissue proper of the mucosa showed a high degree of cellular infiltration and hemorrhage. In other sections the epithelium was missing, and the polymorphonuclears predominated among the cellular elements of the mucosa. Strips of cellular infiltration penetrated into the submucosa.

The liver also showed various degrees of pathologic changes. Small aggregates of leukocytes were scattered throughout the section, located chiefly on the periphery of the acini and at times suggesting direct connection with the small branches of the bile ducts. Occasional comparatively large necrotic areas were encountered. They had a distinct hemorrhagic halo and were surrounded by a zone of cellular infiltration in which the polymorphonuclear leukocytes were predominant. Large deeply stained bodies were sometimes encountered in the peripheral zone of these foci, of a questioned parasitic nature.

It was impossible to decide in how great a degree the changes described were due to infection by cholera vibrios. Some of the lesions in the liver were of too chronic a character to be primarily caused by the cholera vibrios in so short a time. Nevertheless, the presence of cholera vibrios in the smears and cultures from the diseased parts of the liver indicates that these organisms played a part in producing the lesions. They may have found a locus minoris resistentiae in the pre-existing lesions. Except in animals dying of general infection shortly after inoculation, no cholera vibrios were found in the blood, peritoneum, spleen, or lung. In the liver they were found occasionally.

An attempt was made to estimate the number of the cholera vibrios in the various sections of the alimentary canal.

Smears from the gall-bladder, duodenum, and ileum, were examined under the microscope. Equal and constant amounts of the contents of the gall-bladder, duodenum, ileum, and cecum were smeared directly on Dieudonné and plain alkaline-agar plates. The Dieudonné plates were intended to show the number of cholera colonies exclusive of other intestinal bacteria; the plain agar plates, the number of bacteria other than cholera vibrios in the digestive system of the animals; and the microscopic examination of the smears was to demonstrate the presence of any bacteria which might refuse to grow on the plates, whether on account of the alkaline reaction of the media or aerobic methods of cultivation.

The vibrios were almost constantly present, occurring in greatest numbers in the gall-bladder and the ileum. The duodenum and the cecum sometimes contained numerous cholera vibrios; at other times none. The number found in the various parts of the alimentary canal varied greatly with individual animals.

The following tables show also that cholera vibrios are practically the only bacteria present in the proximal part of the alimentary canal of experimental cholera-carriers during life. Smears and plate cultures directly from the bile, and from the contents of the duodenum and the ileum showed cholera vibrios in pure culture in every case in which autopsy and bacteriologic examination were made immediately after the death of the animal. In our first paper we stated that the large intestine, including the cecum, harbored bacteria grossly resembling vibrios; microscopic examination of the contents of the cecum was therefore disregarded. These micro-organisms and fusiform bacilli were encountered frequently, as well as micrococci and rod-shaped bacteria in the smears and peptone cultures, but not on the Dieudonné agar plates. Plain agar plates inoculated directly from the cecum showed at times remarkably few colonies other than cholera vibrios.

Under normal conditions, then, the cholera vibrios greatly predominate in the proximal part of the alimentary canal; the competition with other intestinal bacteria begins to take place in the large intestine. This factor, as well as the consistency of the contents in the large intestine, is responsible for the irregularity with which the cholera vibrios appeared in the feces of the experimental cholera-carriers.

The number of cholera vibrios in the gall-bladder and in the intestine, as before stated, varied considerably with individual animals. In some cases the cholera vibrios were evidently merely passed from the gall-bladder into the intestine, while in other instances they were present in the intestine in great numbers as tho they had multiplied in the intestine. Among the numerous guinea-pigs examined not a single animal was encountered in which cholera vibrios were found only in the gall-bladder and not in the intestine; in other words, there was no evidence that the inflammatory process in the gall-bladder had arrested the function of the organ. All guinea-pigs examined within 14 days after inoculation harbored cholera vibrios in the gall-bladder and in the intestine. Those guinea-pigs which were examined later than 14 days after inoculation, and were found negative, showed eventual signs of inflammation of the gall-bladder, but the organ was maintaining its function; that is, free communication evidently existed between the gall-bladder and the intestine.

EXPERIMENTS ON RABBITS

The following experiments cover the duration of the carrier state and the nature of the condition in rabbits.

Technic

The intravesicular inoculation is technically more difficult in rabbits than in guinea-pigs because the anatomic conditions make it necessary to open the peritoneal cavity by a large incision, and to perform the injection proper within the peritoneal cavity. The gall-bladder in rabbits is oval in shape, firmly adherent to the liver; the cervix is buried in the liver tissue. The cystic duct branches off on the side of the distal end of the gall-bladder. Thus the cervix has the shape of a diverticulum. The guinea-pig's gall-bladder is pear-shaped, narrowing gradually towards the cystic duct. These anatomic differences are probably responsible for the difference in behavior between guinea-pigs and rabbits toward the intravesicular inoculation of cholera vibrios.

The inflammatory process of the gall-bladder which follows the intravesicular injection of cholera vibrios was of much greater intensity in rabbits than in guinea-pigs, bringing about the occlusion of the gall-bladder in the majority of the animals. The communication between the gall-bladder and the intestine was interrupted, so that the intestine was free from cholera vibrios at a time when they were still present in the contents of the gall-bladder.

The gall bladder at this period was usually smaller than normal, and its wall, including the mucosa, thickened. It contained a thin colorless liquid and a creamy sediment, which consisted of pus cells in various stages of degeneration, and of amorphous debris. At times the gall-bladder was filled with thick inspissated pus, composed of pus cells, fibrin, and amorphous debris. In this stage the gall-bladder represents a focus, completely encapsulated by the thickened wall, in which the cholera vibrios remain alive for a certain time, but are no longer passed into the intestine.

TABLE 1
RESULTS OF EXAMINATION OF VARIOUS ORGANS OF EXPERIMENTAL CHOLERA-CARRIERS FOR THE PRESENCE OF CHOLERA VIBRIOS

Guinea-pig	Days After Inoculation	Culture*	Gall-bladder	Duodenum	Ileum	Cecum
69	3	63	+	+	+	+
68	4	63	+	+	+	+
65	5	57	+	+	+	+
78	6	4	+	+	+	+
67	7	63	+	+	+	+
70	8	63	+	+	+	+
66	...	57	+	+	+	+

* In this and the following tables the number of the culture corresponds with the number of the last guinea-pig from whose gall-bladder the culture was recovered.

KEY TO TABLES 1, 2, AND 3

— = cholera vibrios not found.
+ = cholera vibrios present.

TABLE 2
ESTIMATION OF THE NUMBER OF CHOLERA VIBRIOS IN THE VARIOUS SECTIONS OF THE ALIMENTARY CANAL

Guinea-pig	Days After Inoculation	Culture	Dieudonné Plates			
			Gall-bladder	Duodenum	Ileum	Cecum
108	1	4	0	0	0	0
104	2	4	0	0	0	0
111	2	4	0	0	0	0
96	4	57	Num.	—	Num.	Few
97	4	57	Very num.	Num.	Few	Very few
94	4	57	Num.	Few	Very num.	Num.
119	4	4	Very num.	Num.	Very num.	Few
106	6	4	Num.	—	Num.	Very few
107	6	4	0	0	0	0
113	6	4	0	0	0	0
80	7	4	Very num.	Very few	Very num.	Num.
83	7	57	Very num.	Few	Few	Very few
115	7	4	0	0	0	0
112	7	4	Very num.	Very num.	Very num.	Very num.
99	8	57	Very num.	Few	Few	—
100	8	57	Very num.	Num.	Very num.	—
98	9	57	Very num.	Num.	Num.	—

TABLE 3
THE DISTRIBUTION OF CHOLERA VIBRIOS THROUGHOUT THE ORGANS OF INOCULATED RABBITS AND THE DURATION OF THE STATE OF CARRIER

Rabbit	Days After Inoculation	Culture	Direct Plates			
			Gall-bladder	Duodenum	Ileum	Cecum
9	3	4	Num.	Num.	Num.	Num.
14	5	4	Num.	Very few	Few	Very few
11	9	4	—	—	—	—
13	10	4	Few	—	—	—
2	13	4	0	0	0	0
10	13	4	Num.	—	—	—
12	14	4	—	—	—	—
3	57	4	—	—	—	—

TABLE 1—*Continued*

RESULTS OF EXAMINATION OF VARIOUS ORGANS OF EXPERIMENTAL CHOLERA-CARRIERS FOR THE PRESENCE OF CHOLERA VIBRIOS

Liver	Lung	Spleen	Blood	Peritoneum	Remarks
+	+	+	+	+	Died
—	—	—	—	—	Killed
+	+	+	+	+	Died
+	—	—	—	—	Killed. Abscesses in the liver
+	—	—	—	—	Killed. Abscesses in the liver
+	—	—	—	—	Killed. Abscesses in the liver
—	—	—	—	—	Killed

KEY TO TABLES 1, 2, AND 3—*Continued*

Very num. = more than 200.
Num. = about 200.
Few = about 12.
Very few = less than 6.
0 = not examined.

TABLE 2—*Continued*

ESTIMATION OF THE NUMBER OF CHOLERA VIBRIOS IN THE VARIOUS SECTIONS OF THE ALIMENTARY CANAL

Plain Agar Plates				Microscopic Examination		
Gall-bladder	Duodenum	Ileum	Cecum	Gall-bladder	Duodenum	Ileum
Num. Pure	Num. Pure	Num. Pure	Few cholera colonies. Others	+ Pure	+ Pure	+ Pure
Num. Pure	Num. Pure	Num. Pure	Num. cholera colonies. Others	+ Pure	+ Pure	+ Pure
Num. Pure	Num. Pure	Num. Others	Few cholera colonies. Others	+ Pure	+ Others	+ Others
0	0	0	0	+ Pure	+ Pure	+ Others
0	0	0	0	+ Pure	+ Pure	+ Pure
0	0	0	0	+ Pure	+ Pure	+ Pure
0	0	0	0	+ Pure	+ Pure	+ Pure
Num. Pure	Num. cholera colonies. Others	Overgrown by others	…….	+ Pure	+ Others	+ Others
Few	—	Few Pure	— Others	—	—	—
Very num. Pure	Very num. Pure	Very num. Pure	Few cholera colonies. Others	+ Pure	+ Pure	+ Pure
0	0	0	0	+ Pure	+ Pure	+ Pure
0	0	0	0	+ Pure	+ Pure	+ Pure
Very num. Pure	Num. cholera colonies. Others	Num. Pure	Num. Pure	+ Pure	+ Pure	+ Pure
Very num. Pure	Very num. Pure	Very num. Pure	Very num. Pure	+ Pure	+ Pure	+ Pure
0	0	0	0	+ Pure	—	+ Pure
0	0	0	0	+ Pure	+ Pure	+ Pure
0	0	0	0	+ Pure	+ Pure	+ Pure

TABLE 3—*Continued*

THE DISTRIBUTION OF CHOLERA VIBRIOS THROUGHOUT THE ORGANS OF INOCULATED RABBITS AND THE DURATION OF THE STATE OF CARRIER

Peptone Cultures								Remarks
Gall-bladder	Duodenum	Ileum	Cecum	Liver	Lung	Spleen	Peritoneum	
+	+	+	+	+	—	—	—	Abscess in the liver. Dead
+	+	+	+	—	—	—	—	Killed
+	—	—	—	—	—	—	—	Killed
+	—	—	—	—	—	—	—	Killed
+	—	—	—	—	—	—	—	Killed
+	—	—	—	—	—	—	—	Killed
—	—	—	—	—	—	—	—	Killed
—	—	—	—	—	—	—	—	Killed

CONCLUSION

Sufficient evidence has been gathered, it is believed, to justify the opinion that the condition in question is an infection of the gall-bladder, and that the cholera vibrios injected into and recovered by culture from the gall-bladder, stand in causal connection with the pathologic changes encountered, instead of merely surviving as saprophytes at the place of inoculation.

The infectious process may vary in extent and intensity; remain limited to the gall-bladder, or extend to the liver.

The absence of cholera vibrios from blood from the lungs, and from the spleen makes the septicemic character of the infection highly improbable.

With regard to the intravesicular inoculation the rabbit's behavior differed from that of the guinea-pig inasmuch as the inflammatory process which followed the injection of cholera vibrios brought about the occlusion of the gall-bladder so that the cholera vibrios were no longer to be found in the intestine at a time when they were still present in the contents of the gall-bladder.

It is evidently a benign process which shows marked tendency to healing; i. e. the cholera vibrios disappear from the animal's body and the animal survives, altho a large percentage of the animals showed signs of chronic intoxication.